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THE METHODS EMPLOYED AT THE NAPLES ZOOLOGICAL STATION FOR THE PRESERVATION OF MARINE ANIMALS.

BY

DR. SALVATORE LO BIANCO.

Translated from the original Italian
BY
EDMUND OTIS HOVEY.

Part M of Bulletin of the United States National Museum, No. 39.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1899.

USNM

work rooms. The smaller building is used for receiving, preserving, and storing the material brought in from day to day, and for laboratories and study rooms for some of the officials and others. The aquarium proper contains twenty-six tanks having glass fronts and lighted from above in such a manner that, as a person gazes into them, he can readily imagine himself standing on the bottom of the sea with the animals at home about him. There are no open tanks and no light enters the room except that which comes through the water.

The stocking of the aquarium and the supply of material for the naturalists at work in the station is cared for in a very complete way. The station owns two steam launches, which are used for dredging and other heavy or distant work, and two or more small rowboats, which are kept constantly busy collecting jelly-fish and other surface forms of life which are driven into the harbor from the open sea. Furthermore, all the fishermen in the bay regularly bring to the station all the animals that come up in their nets that are known to be desired. These objects are paid for as they are brought in. Every day the naturalists state what they need for the next day's work, and every evening corresponding orders are given to the crews of the boats belonging to the station. One naturalist may want fifty sea-urchins of a kind, another twenty-five starfish, another a large or small number of jelly-fish or crustaceans or what not, and each finds his wants supplied the next morning, if the weather has been favorable. It is very interesting to observe the pride which the rough fishermen of the bay now take in bringing to the station all rarities and other forms of life which they know to be desired there.

Although the aquarium is the most popular portion of the institution and the public sees only that and the preserved animals which are sent out to museums, the chief mission of the station, in the eyes of the director, Dr. Dohrn, and of all other scientists as well, is to provide a place for the investigation of marine life under the best conditions attainable on land, and most of the station building is given up to provisions for this purpose. The regular scientific corps consists of nine men, including the director, each of whom makes a special study of some form of animal life. The average number of naturalists not connected with the institution who study there is about forty in each year, though not more than from twenty-five to thirty may be there at one time. Each of these is an independent worker along some particular line of study, no elementary instruction being given by the officers. Each student is provided with a table or desk, drawers, racks, book-shelves, microscope, glassware, alcohol, and other reagents, drawing materials, glass tanks with running and stationary water, and, in fact, with everything needed to carry on his investigations, and with animals to work upon.

The library is very full on all subjects bearing upon zoology, a specialty being made of periodicals. Furthermore, fully equipped laboratories are provided for the investigation of the chemical and

physical questions which arise, and there are optical and photographic rooms, and a machine shop fitted up for doing all ordinary grades of work. More than a thousand naturalists have availed themselves of these opportunities for study. Of this number 375 have been Germans, 240 Italians, 85 Russians, 80 Englishmen, 40 Dutchmen, 40 Americans (from the United States), 36 Swiss, 35 Austrians, 22 Belgians, 17 Spaniards, 17 Hungarians, the remainder being Scandinavians, Greeks, Roumanians, Bulgarians, Japanese, and Hindoos. Truly an international array!

The activity of the investigators at the station does not expend itself wholly or even largely upon the description and naming of new species. It seeks rather to discover the innermost secrets of life, and to learn all the comparisons that can be made between one set of animals and another. The results of the work are published in three ways and appear periodically. The description of the Fauna and Flora of the Gulf of Naples began to be published in 1880, and up to the end of 1897 twenty-four quarto volumes of these exhaustive memoirs had been issued, magnificently illustrated with numerous text fixures and colored and plain plates. The Contributions from the Zoological Stations are the shorter articles brought out by the workers at the station, and thirteen volumes of these have appeared. The Zoological Yearbook is now in its twenty-first volume, and seeks to give not only a list of all articles and books of the current year pertaining to the science, but also brief abstracts of their contents.

To inaugurate the station in 1872 required about $100,000, besides the land in the beautiful Villa Nazionale donated by the city of Naples. Friends of science in Germany and England contributed about $40,000 of this amount, but the remainder came from Dr. Dohrn's own fortune. The money necessary to meet the running expenses of the institution comes from several sources. Each contributor of £100 annually to the station supports a "table," and has the right to name a person to receive the benefits thereof. At present 30 tables are thus provided for, the Italian Government paying for 7, different institutions in Germany for 11, England for 3, Russia, Austria, and the United States[1] for 2 each, and Belgium, Holland, and Switzerland for 1 each. The tables are paid for year by year, and there is no endowment fund, though Dr. Dohrn is striving now to establish one. The German Government appropriates £2,000 for the station; the fees of visitors to the aquarium amount to about £1,000; the sale of preserved animals to about £700, and the sale of old material of various kinds to about £100. The expenses, however, always manage to keep pace with

[1] The Smithsonian Institution has supported a table at the Station regularly since 1893. The Collegiate Alumnæ have begun supporting a "woman's table" in commemmoration of the twenty-fifth anniversary of the Station. During some years Columbia University has had a table, and one has been paid for at intervals by a few of the leading American universities alone or in conjunction with the American Society of Naturalists.

the income, or exceed it, although the officers of the institution work for almost nothing, the highest salary paid being less than $1,100 a year.

The branch of activity at the station which appeals more strongly to the public than do the scientific investigations is the preservation of marine animals, particularly invertebrates, for exhibition in museums and for purposes of study. To Cav. Dr. Salvatore Lo Bianco is due the major part of the credit for the success which the station has had in this line of work. Beginning work for the station as an attendant, he soon showed peculiar ability in handling the animals which were obtained. He studied their habits, anatomy, and composition carefully, and made many experiments to determine the best method of killing and preparing each species, so that it would present a lifelike appearance in the exhibition jar, until he succeeded in obtaining the beautiful results which have been distributed to museums and colleges all over the world. The animals which are intended for dissection and study must often be preserved in a different manner from those which are intended for exhibition, and in this line of work, also, the Naples station stands preeminent. An investigator may even send word to have specimens prepared in special ways of his own devising, and thus save himself the trouble of making a trip to Naples.

One reason for the beautiful appearance of the material sent out by the station is that it is properly caught in the first place; another is that, for the most part, the animals are alive when the process of preservation begins. With many forms it is indispensable that they be alive at the beginning of operations; with some it is not so necessary, but with all it is highly desirable. A fish which has been put into alcohol after death looks entirely different from another specimen of the same species which has been put into the fluid when still alive. The best methods have been determined for each species by itself, different species of the same genus often requiring different handling, hence it is necessary that the operator should be able to recognize the species with which he has to deal in order to obtain the best results. When new species are encountered, the best mode of precedure must be determined by experiment. The experience of the Naples station has been so long and varied, however, that a knowledge of the methods pursued there will be of value to naturalists all over the world. For this reason the writer, with the consent of Professor Dohrn and the consent and cooperation of Dr. Lo Bianco, has undertaken the translation of the latter's "Metodi usati nella Stazione Zoologica per la conservazione degli animali marini,"[1] incorporating therewith the notes made during a stay of five weeks at the station in the autumn of 1897 for the express purpose of studying those methods for the benefit of the American Museum of Natural History. It is to be understood that the methods have all been devised by Dr. Lo Bianco, unless otherwise stated.

EDMUND OTIS HOVEY.

NEW YORK, *March, 1899.*

[1] Mittheilungen a. d. Zoologischen Station zu Neapel, Pt. 3, IX, 1890, pp. 435–474.

THE METHODS EMPLOYED AT THE NAPLES ZOOLOGICAL STATION FOR THE PRESERVATION OF MARINE ANIMALS.

By Dr. SALVATORE LO BIANCO.

[Translated from the original Italian by Edmund Otis Hovey.]

UTENSILS.

The laboratory of the station is provided with large tanks containing running and stationary sea water, a table covered with sheet lead and furnished with a drain, a great variety of glass and earthenware dishes, and tools of different kinds and materials.

Cylindrical glass jars, with glass stoppers ground to fit, are used for exhibition purposes and for storage. Those with necks are employed for the most part, but those without necks and with a flat top are preferred for elegant installation. Cylindrical jars are the most economical of fluid and are the cheapest to get.[1] Since glass jars are expensive, earthenware jars and basins are used for many laboratory manipulations. The small, globular vessels which have the bottom formed by a glass stopper, concave within, are recommended for small spherical animals. Round-bottomed glass tubes are very useful, but care must be exercised to see that the walls are not too thin. The edge of the orifice should be smoothed in the Bunsen flame. When the tubes are more than 30 mm. (1.2 inches) in diameter, the lip should be flared out so that a piece of bladder can be readily tied over the opening.

Corks should be selected from the best stock, should be as compact as possible, and should be without cracks or other defects. In form they should be cylindrical, so as to make a good joint with the sides of the tube. The ends must be flat, with clean cut edges, so that no fragments can get into the alcohol. With large tubes it is desirable to put a plug of cotton inside the tube next to the cork, since the alcohol extracts the tannic acid from the cork and is stained brown thereby.

[1] For convenience in suspending objects in the liquid, those having a glass hook in the under side of the stopper should be obtained. E. O. H.

To preserve small, delicate animals, such as eggs and larvæ, it is well to place the small tube containing the objects in alcohol, closed with a cotton plug, inside a larger vessel, which is likewise filled with alcohol. This arrangement prevents danger from evaporation and minimizes the liability to breakage. One must see, however, that the cotton contains no acids and does not stain the alcohol. Absorbent cotton is the most suitable, of course, but the best quality of ordinary cotton will answer every purpose.

For large, flat objects, such as Asterids, Pleuronectids, and the like, rectangular jars with flat sides are recommended. These jars are made to be closed with a plate of glass, cemented on. Gutta-percha cement is generally used. Such receptacles have the great advantage that they do not distort the view of the object within them. For delicate forms, which are long and stiff, like *Funiculina*, glass tubing of proper size, cut off at the right length, is used, one end being closed in the Bunsen flame and the other with a cork.

For preliminary manipulations much use is made of glass crystallizing dishes with flat bases and perpendicular sides, in which many specimens can be placed in little liquid without touching or interfering with one another. They are especially advantageous for keeping animals alive in sea water, letting them remain at rest until thoroughly distended; for killing by different methods, either slowly or quickly, and for hardening objects in different solutions until they are transferred to permanent receptacles. These crystallizing dishes have ground edges so that they may be tightly covered with disks of glass, when desirable. For hardening worms and other elongated animals use may be made of long rectangular vessels covered with a sheet of glass, or of the zinc trays to be described later.

It is also necessary to have a number of ordinary beakers (or battery jars) of different sizes, which serve for the preserving of animals alive, tubes for the reception of small animals, pipettes for the extraction of minute forms from jars of water, glass rods, reagent bottles, graduated cylinders, etc.

For preserving animals, especially fish, of a size too great for such glass receptacles as have been mentioned, a rectangular case or box of zinc with a shallow trough around the margin is very useful. The cover, likewise of zinc, has its edge made to fit into the trough. To prevent evaporation the trough may be filled with water and a layer of oil. The cover has an opening in the middle to permit the escape of the air which is compressed under it by closing the box. This opening is provided with a cork. It must be acknowledged that these boxes have the disadvantage that, after a time, the zinc becomes corroded, probably by some acid formed in the alcohol through the action of dead animal matter. It is a good plan to protect the metal box by an exterior wooden case.

In place of the rectangular vessels of glass for hardening animals of elongated form, the station uses some made of zinc with a layer of wax

in the bottom. The wax bottom is for the purpose of holding the wooden pins used in straightening out worms while they are hardening. Pins of orange or other hard wood are preferable to those of metal, because the fixing fluids attack metals. A convenient size for such a tray is 60 by 6 by 6 cm. (24 by 2½ by 2½ inches) with about 1 cm. (one-half inch) of wax in the bottom.

For the transfer of objects from one receptacle to another spatulas are largely used. These are made preferably of horn, because that material is not attacked by the reagents in use. They range in size from 6 mm. (one-fourth inch) to 10 cm. (4 inches) in width, and are of a convenient length, say 17.5 to 20 cm. (7 or 8 inches).

A pair of soft iron forceps 30 cm. (12 inches) long is very convenient for taking objects out of deep receptacles. Iron is both cheaper and just as good as brass for that purpose. Small forceps, wire cutters, syringes, and so on are used at times.

The apparatus for narcotizing certain Actinians is constructed as follows: The nose of a pair of bellows is provided with a metallic bowl which fits over the metal bowl of a tobacco pipe. The latter is provided with a peg which fits into a slot in the bowl on the bellows and fastens the two together. The tube of the tobacco pipe is continued with a piece of flexible rubber tubing which terminates in a U-shaped piece of glass tubing, the distal end of which has been drawn out to a point. With this apparatus one can easily force smoke into a receptacle.

REAGENTS.

Alcohol.—Without doubt the most indispensable liquid is alcohol. For the preparation and preservation of delicate, transparent animals it is necessary to use purified spirit which has been filtered and diluted with distilled water. For coarser animals ordinary alcohol may be used, if desired, even that which has been obtained by redistilling what has once been used being available, care being exercised to see that acids and alkalies have been neutralized. The station always has on hand a quantity of alcohol of 70 per cent strength, which is what is ordinarily used for preserving animals, that of 90 per cent being used only in special cases. By mixing the alcohol and water somewhat in advance of actual need one avoids the innumerable bubbles of air which form on the surface of an animal when immersed in freshly diluted alcohol. Soft or gelatinous animals must be allowed to remain from two to six hours in alcohol of 35 to 50 per cent, according to their consistency, and then be transferred to that of 60 per cent, and afterwards to that of 70 per cent. If the preparations are too delicate to bear handling, the transfer may be made by pouring off the liquid and adding the proper amount of alcohol to make a 35 per cent solution, continuing the process until the standard strength is attained. When necessary to avoid disturbing the animal at all a siphon may be used in effecting the transfer. Frequently it is necessary to change the alcohol after a few days, on account of discoloration. Some forms are

immersed directly in 70 per cent alcohol, the liquid being changed after a few days. Changes should be made until the alcohol remains colorless. When an animal which has been in alcohol is transferred to that which is stronger, it is necessary to agitate the jar from time to time to avoid the formation of a layer of weaker alcohol on the bottom.

Many liquids have been tried at the station in search for a possible substitute for alcohol, but always with poor results. Some liquids, like those of Goadby and of Owen, when used on gelatinous forms, eventually produce contraction and consequent distortion. Wickersheimer's solution, which was highly praised when first brought out, distorts or macerates marine animals. Alcohol of 70 per cent is preferable for the permanent preservation of animals for the reason that it is sufficiently absorbed by the tissues after repeated changes. A stronger solution not only is unnecessary for good preservation in the majority of cases, but it is even harmful in some, because it eventually hardens the objects too much and renders them brittle. Alcohol is useful, furthermore, for narcotizing animals and for killing them slowly or quickly.

Formalin or formaldehyde.—Formalin is a very useful liquid for keeping animals temporarily, but not for preserving them permanently. Some pelagic animals—for example, certain Medusæ, Pterotrachaidæ, and Salpidæ—may remain in it for even two or three years without serious detriment, but if they are not transferred to alcohol by that time they begin to disintegrate or decompose. Formalin therefore may be used on a voyage or a long journey when alcohol is scarce or not to be had. As a provisional fluid it is useful for many other animals which are not contractile, and especially for those which contain no lime spicules, skeleton, or shells. Shell-bearing mollusks, echinoderms, and such things can not be preserved in formalin on account of the free acid[1] in the fluid, which attacks the calcareous portions and causes them to lose form or brilliancy, or both. In the case of large animals, such as fish, one must make an injection through the anus of a solution of at least 5 per cent strength. With formalin, as with other preservatives, only one, or at any rate only a very few, objects should be put into the same receptacle at the same time, and there must be a good amount of fluid in proportion to the animal mat ter present. For gelatinous animals the solution should be of 1 to 4 per cent strength. *Carmarina* and similar things may be killed and hardened at the same time by the use of formalin of the right strength and chromic acid of 1 per cent in equal parts. For animals of some consistency, like ascidians and fish, one should use a 2 to 6 per cent formalin solution, the general rule being that the softer the animal the weaker the formalin. Either fresh or salt water may be used in making the solutions, as may be convenient. The sea-water solution, indeed, preserves the transparency of gelatinous bodies better than the

[1] It is said by the advocates of the use of formalin that this free acid may be neutralized by sodium carbonate and many of the objections to the fluid thus removed. E. O. H.

other. It is not necessary to wash objects which have been in formalin before transferring them to alcohol. For killing and hardening *Rhizostoma*, *Tima*, and some other animals formalin is excellent, and the objects may remain in the fluid a long time before they are transferred to alcohol. It is readily perceived that the contractile animals, when they have been narcotized by one of the usual methods, may be temporarily preserved in formalin in case alcohol is lacking. Colors certainly are preserved for a longer time in formalin than in alcohol, but in time those which are fugitive in one disappear in the other also. The preservative medium has not yet been discovered which will permanently preserve the colors which are due to a pigment in the skin or substance of an animal.

Chromic acid.—Next to alcohol an aqueous solution of chromic acid is the most useful reagent, and it serves especially for killing and hardening gelatinous and soft animals. Objects, however, should not remain in the fluid longer than is necessary, because they become too deeply tinged and are rendered fragile.

After treatment with the acid it is necessary to wash the animals with fresh water to avoid the formation of a precipitate when they are placed in alcohol. If they are not well washed, they will acquire in time a greenish hue. Chromic acid is used mixed with osmic, acetic, or picric acid, with corrosive sublimate ($HgCl_2$), and rarely with alcohol. The solutions are made in ordinary fresh water when possible, though occasionally salt water may be used. They will not keep long. That which has served once may be used again if it will not be too dilute when added to the water containing the animal and if too much time has not elapsed. When the solution has turned green after standing it is not fit to use.

Acetic acid.—This is a reagent which has the property of permeating tissues instantly and hardening them, and it is a very efficacious means of rapidly killing contractile animals, but it has the disadvantage of softening them again if they remain in it too long a time. Objects remain relatively transparent. In certain cases it is necessary to use a concentrated solution of the acid. It is often mixed with chromic acid for killing and hardening noncontractile transparent animals.

Osmic acid.—In general, osmic acid is not used as much now as formerly, because its use has several inconveniences. Efforts have been made at the station to substitute other reagents for it, and in many cases they have been successful. It hardens gelatinous forms well and preserves the transparency sufficiently, but its action is too great eventually. The preparations become dark-colored and are rendered fragile; consequently they should remain only until they have acquired a light brown tint.[1] Before they are transferred to alcohol

[1] Dr. Paul Mayer's method for bleaching objects which have been too much blackened is not practicable for soft animals, since it softens them too much. (Mitth. Zool. Stat. Neapel, II, 1880, p. 8.)

they should be washed for some minutes in fresh or distilled water, as should be done with all animals which have been treated with any mixture containing osmium.

Kleinenberg's liquid[1] was one of the first adopted at the Zoological Station for the preservation of marine forms. Since it presents the disadvantage of staining the alcohol, even after repeated washings, and of not hardening the animals sufficiently, its use has been given up little by little, until now it is confined to the preparation of histological subjects, with the single exception of *Balanoglossus*, which is killed with this solution for exhibition as well as for study.

Lactic acid, in a solution of one in a thousand in sea water, serves well in treating larvæ and small gelatinous organisms.

Hydrochloric, pyroligneous, and *sulphuric acids* are used rarely.

Corrosive sublimate, recommended first by A. Lang, is much used as a fixing agent, because it has the property of permeating tissues rapidly and hardening greatly. It is used in concentrated solution in fresh or sea water, either cold or hot. In manipulations with sublimate, metallic implements must not be used, because they decompose the solution and stain the preparations. The solution is made, when possible, with hot water for economy of time, and in vessels of glass or porcelain. Care must be exercised to avoid boiling the sublimate in open vessels, and not to inhale the vapors. The hands must not be immersed in the solution if they have on them open cuts or sores.

All animals which have been prepared with this reagent can be used for histological researches. Corrosive sublimate is also used mixed with acetic or chromic acid or with sulphate of copper. Animals which have been treated with corrosive sublimate must be washed carefully and thoroughly in fresh water before they are placed in alcohol. Add a solution of iodine drop by drop until the alcohol remains permanently colored thereby; this insures the entire removal of crystals of corrosive sublimate from the substance of the animal. If this precaution is not taken, the mercury will be reduced from the corrosive sublimate and will stain the animal black, forming a black precipitate on the sides and bottom of the vessel. The amount of iodine to be used depends upon the size and character of animal to be treated.

Bichromate of potassium.—This is used as a 5 per cent solution for slowly hardening gelatinous animals without rendering them too fragile, when it is not possible to work with chromic acid. On account of the troublesome precipitate which forms when objects treated with bichromate are transferred to alcohol, the use of this reagent is not recommended. For bleaching the preparations before they are put into alcohol, use a few drops of concentrated sulphuric acid.

Sulphate of copper.—This is used only in solutions from 5 per cent to 10 per cent strength, which are made with hot fresh water, and used

[1] Kleinenberg's liquid is made by mixing 100 c. c. of a saturated aqueous solution of picric acid with 2 c. c. of concentrated sulphuric acid. Filter and add three volumes of distilled water.

alone or mixed with corrosive sublimate for killing larvæ and delicate animals. The objects which have been treated with this reagent must be washed repeatedly with water or else they will not remain perfectly clear, owing to the formation of crystals within the tissues, which render them opaque. If they afterwards prove to have been washed too little, the objects should be treated several times with an acid.

Chloral hydrate.—This is used in very weak solution, from 0.1 to 0.2 of 1 per cent made fresh in sea water, for narcotizing several forms before fixing them. This method has the advantage that, if the animal after a certain time does not remain in the condition desired for preservation, it can be replaced in sea water, where it will regain power of motion and continue to live. It is used for killing animals which live in the crevices of a rock, in incrustations of calcareous algæ, and among colonies of serpulas and of madrepores. Such must be allowed to remain in the solution from six to twelve hours. It is not necessary to use a fine quality of the drug.

Cocaine.—A solution of cocaine is made by dissolving 2 grams of the powder in 100 cubic centimeters of 50 per cent alcohol. This is a most excellent narcotizing medium, but its high cost prevents its extensive use. It is the best reagent thus far discovered for the treatment of gastropods. A few drops are carefully distributed over the surface of the water containing the animals, and the operation is repeated until the animals cease to respond to any stimulus.

Other reagents that are used occasionally are *chloroform*, *ether*, and *tincture of iodine*.

Mixtures frequently used.

Alcohol of 70 per cent and chromic acid of 1 per cent in equal parts.

Alcohol of 50 per cent	cubic centimeters	100
Hydrochloric acid (concentrated)	do	5
Alcohol of 35 per cent or of 70 per cent	do	100
Alcoholic tincture of iodine	do	2.5
Alcoholized sea water:		
Sea water	do	100
Absolute alcohol	do	5
Chrom-acetic mixture No. 1:		
Chromic acid of 1 per cent	do	100
Concentrated acetic acid	do	5
Chrom-acetic mixture No. 2:		
Chromic acid of 1 per cent	do	10
Concentrated acetic acid	do	100
Chrom-osmic mixture:		
Chromic acid of 1 per cent	do	100
Osmic acid of 1 per cent	do	2
Chrom-picric mixture:		
Chromic acid of 1 per cent	do	50
Kleinenberg's solution	do	50
Sulphate of copper, solution of 10 per cent strength	do	100
Corrosive sublimate, saturated solution	do	10

Potassium bichromate, 5 per cent solution	cubic centimeters	100
Osmic acid, 1 per cent solution	do	2
Corrosive sublimate, saturated solution	do	100
Acetic acid, concentrated	do	50
Corrosive sublimate, saturated solution	do	100
Chromic acid of 1 per cent	do	50
Kleinenberg's solution:		
Picric acid, saturated solution	do	100
Concentrated sulphuric acid	do	2
Filter and add three times the volume of distilled water.		
Flemming's solution:		
Chromic acid of 1 per cent	do	25
Osmic acid of 1 per cent	do	10
Acetic acid (glacial)	do	5
Distilled water	do	60
Perenyi's solution:		
Nitric acid of 10 per cent	do	40
Chromic acid of ½ per cent	do	30
Alcohol of 90 per cent	do	30
Müller's solution:		
Potassium bichromate	grams	2
Sodium sulphate	do	1
Distilled water	cubic centimeters	100
Formalin solution (standard):		
Commercial formalin 40 per cent	do	10
Water	do	90

METHODS OF PREPARATION AND PRESERVATION.

PROTOZOA.

Protozoa are so small for the most part as to be invisible to the unaided eye, and their preparation therefore comes within the field of the microscopist, for which reason only the larger species will be mentioned here. Certain Gregarinas which occur as parasites in the intestinal nucleus of *Salpa maxima-africana* are best preserved with Kleinenberg's solution, where they may remain for about an hour before they are transferred to weak alcohol.

RADIOLARIA.

Thalassicolla is well fixed in chromic acid of ½ of 1 per cent, where it may remain about an hour and then be transferred gradually to alcohol of 70 per cent.

Aulacanthidæ and Acanthometræ are placed directly in 50 per cent alcohol, and after a few hours are transferred to that of 70 per cent. Good preparations have also been made by dropping a few drops of 1 per cent osmic acid into the sea water containing the animals and then transferring them to alcohol after washing them in fresh water. Excellent microscopic preparations of some of these species of minute pelagic organisms have been made by a treatment with a saturated solution of corrosive sublimate.

Sphærozoidæ.[1]—The different species of the genera *Sphærozoum* and *Collozoum*, which have spherical or cylindrical form, are fixed in iodinized alcohol of 35 per cent, where they should remain from fifteen minutes to about an hour. The vessel containing them should be shaken from time to time, because the animals flatten out if they are allowed to remain too long on the bottom. If it is desired to prepare a large number at one time, it is necessary to put the fixing liquid into a crystallizing dish of ample size, for convenience in manipulation. After a sufficient time they are transferred to alcohol of 35 per cent, where they can remain a few hours. The change is effected by transporting the colonies with a spatula to another crystallizing dish of the same size without allowing the animals to be without liquid. In the same manner they are transferred to alcohol of 50 per cent, and after twelve hours to that of 70 per cent, and the last should be renewed after twenty-four hours. In this manner colorless preparations are obtained which can also serve for histological studies. Osmic acid is not recommended, because it darkens the preparations too much.

In colonies of *Sphærozoum* with isosporic structure the shape is not fixed with iodinized alcohol, and it is necessary to use saturated sublimate. The genera *Myxosphæra*, *Acrosphæra*, and *Collosphæra* are killed in chromic acid of 1 per cent, to which a few drops of osmic acid have been added, using the same form of receptacle and the same precautions mentioned under *Collozoum*. After from half an hour to an hour the acid solution should be poured off and fresh water substituted for washing, but great care must be exercised not to break the colonies. Then the objects are gradually transferred to alcohol.

Acinetidæ.—*Trichophrya salparum* has yielded beautiful microscopical preparations when treated with concentrated sublimate in sea water. With *Acineta fœtida*, which usually lives among hydroids, better results can be obtained with osmic acid.

Vorticellidæ.—The colonies of *Zoöthamnium* are best killed with boiling saturated sublimate.

PORIFERA.

For sponges which are to be used for exhibition, it is enough to immerse them directly in 70 per cent alcohol, renewing it when it becomes discolored. To avoid the contraction of Halisarcidæ, they should be fixed in chromic acid of 1 per cent for about half an hour, or in saturated sublimate for fifteen minutes. Those sponges which are to serve for study, if they are not too large—that is, if they are not more than 10 cm. (4 inches) in diameter—are immersed in 90 per cent alcohol or in absolute alcohol, which should be renewed after three or four hours, and again after twenty-four to forty-eight hours. If the

[1] These methods are described in full by K. Brandt on pp. 7–11 of his monograph: Die Koloniebildenden Radiolarien (Sphærozoïen) des Golfes von Neapel, in Fauna Flora Golf Neapel, XIII, Monograph, 1885.

specimens are too large, small pieces may be cut off and treated in this manner.

If they are to be dried, they should be washed first in fresh water for a few hours, then they should lie for about a day in ordinary alcohol, and then be placed in the air or in the sun. If treated in this way, they will not have an offensive odor. If it is desired to retain the rosy color of certain sponges (*Suberites*, *Axinella*) for several days, it is enough to place them in 40 per cent alcohol and not change it.

ANTHOZOA.

The first thing to be done when an Anthozoan has been caught is to place it in a receptacle with fresh sea water. It always happens that these animals, when disturbed by the fishing apparatus or transportation, contract or withdraw into themselves completely. To cause them to expand, it is enough to let them remain in a jar with pure sea water, although it may be necessary to keep them for a longer or shorter time in running water. Many times it has been noticed that the water soon becomes bad if it is not changed.

The following methods, especially that with the chrom-acetic mixture No. 2, are used for preserving animals for museums and to some extent for the study of gross anatomy.

Since all the Alcyonarians contain minute calcareous spicules which furnish the specific characters, they should remain in the acid mixture as short a time as possible so that the acid may not attack the spicules.

In those cases in which chrom-acetic mixture No. 2 has not given good results, a mixture of sublimate and acetic acid may be employed, but always for the killing alone. The animals should be transferred quickly to weak alcohol.

A method used by G. von Koch is quickly to immerse the distended animals in absolute alcohol or that of 90 per cent, making an injection of the same afterwards into the interior of the animal.

When the colonies of *Cornularia*, *Clavularia*, *Rhizoxenia*, and *Sympodium* have become expanded, siphon off the water in the receptacle until only enough remains to cover them. This operation should be performed with great care to avoid any shock which could cause the retraction of the tentacles. Then pour rapidly into the jar a volume of chrom-acetic No. 2 double that of the water in which the animals remain, and immediately afterwards transfer them to alcohol of from 35 to 50 per cent, giving the preparation a few gentle shakes to free the tentacles and dispose them in natural manner. Another good method of killing is with hot saturated sublimate, using the same proportions as of the chrom-acetic mixture, and washing the animals when scarcely dead in fresh water before the transfer to weak alcohol.

The large *Alcyonium* is treated in the following manner: After the rapid bath in chrom-acetic No. 2 it is suspended, scarcely dead, in a jar containing weak alcohol in such a way that its tentacles do not touch the

walls of the jar, and if the polyps remain well distended, the change to the different grades of alcohol then goes forward very gradually. In the weak alcohol minute bubbles of air frequently form and attach themselves to the tentacles, giving them a tendency to float and thus causing distortion. Striking the sides of the receptacle with light blows will rid the tentacles of the bubbles.

Pennatula phosphorea and *Kophobelemnon*, when they have become well distended, are taken by the naked base and very swiftly immersed in a tall, cylindrical jar containing the chrom-acetic mixture No. 2, and after a few seconds are placed in a crystallizing dish containing 50 per cent alcohol, where they are allowed to rest on their backs. Then with a small syringe with a very fine point inject alcohol into them through a minute hole made in the extremity of the base. In this manner the alcohol penetrates to all parts of the interior and distends the polyps. Tie a thread around the end of the base above the hole which was made so that the escape of the alcohol may be prevented. After some hours the animals should be transferred to 70 per cent alcohol, and in the final receptacle *Kophobelemnon* should be suspended upside down by means of a glass float with a hook in it.

Pennatula rubra, *Pteroides spinulosus*, *Veretillum*, and *Funiculina* are killed like the Pennatulids just mentioned, but no injection is made after the transfer to weak alcohol. Soft forms like *Veretillum* should be suspended in the final receptacle. Small forms of the Pennatulids may be killed without removing them from the vessel in which they have become distended, and they are then treated like the Cornularians.

Gorgonia, *Gorgonella*, *Primnoa*, *Muricea*, *Isis*, etc., should be killed with chrom-acetic mixture No. 2 in the same dish in which they have become distended on account of the great sensitiveness of their polyps. It is always advisable to have as little water as possible in the dish at the time of killing these animals, and to pour over them a volume of the mixture twice as great as that of the water in which they are. Several times it has been noted at the station that the Gorgonidæ which have expanded in sea water which has begun to turn bad are those which have given the better preparations. The small colonies, or pieces of colonies, remain with their polyps distended if they are killed with boiling saturated sublimate. *Isis* may be well preserved by using a mixture of sublimate and acetic acid.

Corallium rubrum, after it has been allowed to expand in running sea water, should be killed with boiling saturated sublimate solution (half as much as the water containing the coral), and quickly transferred to weak alcohol. By this method the color is almost perfectly preserved, while by the use of the chrom-acetic mixture it is very much injured. The alcohol which has been used for this coral can not well be used afterwards for the preparation of any delicate organism. A colony of *Antipathes* which was placed in such alcohol was dyed red within twenty-four hours.

ZOANTHARIA.

All the species of *Antipathes* are fixed with saturated sublimate, and, on account of the slight contractility of the polyps, their preparation always succeeds. The saturated sublimate, which is used cold, should be of the same volume as the water containing the animals.

ACTINIARIA.

The preparation of this group is very difficult, the great contractility and resistance of the muscular system of the majority of the species frequently constituting insurmountable obstacles to success. Many times when it is thought that the animal has been deprived of any sensitiveness, immersion in a reagent of rapid action is sufficient to show sudden and surprising contraction of the tentacles and of the whole body. When several specimens of certain forms are treated with the same method and under the same conditions, some die distended and the rest contracted. Good results depend, in some cases, on circumstances which, up to the present time, are wholly unknown. After all, however, there are many species with which perfect results can be attained, if great care be exercised in the manipulations.

Anemonia sulcata (*Anthea cereus*) is the easiest to prepare. When well distended in running water, the animals are killed with the chrom-picric solution, used in volume equal to that of the water in which they are. This should be rapidly poured into the jar containing the Actinian, after as much of the water therein has been poured off as may be and leave the animal immersed. A solution which is now much used instead of the chrom-picric mixture just mentioned is made of—

Chromic acid, of 1 per cent	1 part
Saturated solution picric acid	1 part
Formalin, of 4 per cent	1 part

When the animals die, they will fall from the sides of the glass, and they should then be transferred to another jar containing chromic acid of one-half per cent, where they should be suspended upside down by means of a glass float, the hook of which has been passed through the lower rim of the body. The animals should be gently shaken to give the tentacles a natural position. After half an hour they are placed in weak alcohol, and then gradually transferred to that of 70 per cent. It is a good plan to suspend the animals upside down by means of a float in the final receptacle, though it is hardly worth while to do this for the smaller specimens.

The following Actinians may be killed with boiling saturated sublimate: *Eloactis*, *Sagartia dohrni*, *Paranthus*, *Corynactis*, and small specimens of *Aiptasia*. Before they are transferred to alcohol, the animals should be allowed to harden for some minutes in chromic acid of one-half per cent.

When *Heliactis bellis*, *Bunodes gemmaceus*, and *B. rigidus* are well distended, two-thirds of the sea water in the jar containing them should be removed and its place filled by a chloral hydrate solution 0.2 of 1

per cent. After a few minutes this liquid should be poured off until there is barely enough in the jar to cover the animals, which should then be killed with cold saturated sublimate.

Adamsia rondeleti is narcotized with tobacco smoke in the following manner: Remove the hermit crabs from the shells on which the actinias are growing and kill them in fresh water. Suspend the shells by threads in beakers of sea water in which the actinias will have ample room for expansion. The thread may be wound around the shell or passed through a hole in it and then tied over a stick of wood which rests on the edge of the beaker. Place one or more of the beakers in a shallow tray (preferably with flat bottom and perpendicular sides) containing a little water and cover with a bell glass. Fill the bell glass by means of the bellows and pipe described on page 9, being careful at the same time to insert a U-shaped piece of glass tubing under the edge of the bell glass to permit the escape of the confined air as the smoke is forced in. Avoid jarring the glasses containing the actinias.

To regulate properly the duration of the whole operation it is necessary that the first fumigation should be made about 2 o'clock in the afternoon. Little by little the smoke clears up and the water begins to absorb the narcotizing substances contained therein and the animals for the most part distend the corona of tentacles. About 5 o'clock a second fumigation like the first should be made, and the objects are allowed then to remain overnight. The following morning carefully remove the bell jar and touch the tentacles with a needle to learn in what condition of sensibility they are. If they do not contract under this stimulus, place a small open beaker containing a few cubic centimeters of chloroform beside the jar containing the actinia and replace the bell jar, allowing the fumes of the chloroform to work for two or three hours. Lastly the animals are killed in the chrom-acetic mixture No. 2, hardened with chromic acid of one-half per cent and placed in weak alcohol and so on, where they are to remain suspended. If the tentacles give signs of sensitiveness, make a third fumigation and after a few hours test again. This is the only method which has proved successful in obtaining specimens with the column well distended and with the disk and tentacles in full expansion. Cold weather retards this and other narcotizing processes in a very marked degree.

Adamsia palliata can be prepared in the same manner without suspension. Good results have also been obtained by narcotizing the animal slowly with alcoholized sea water and then killing with the chrom-acetic mixture No. 2, or with hot saturated sublimate.

Cladactis, *Cereactis*, and the little *Bunodeopsis strumosa* are killed with the chrom-acetic mixture No. 2, and immediately afterwards hardened in chromic acid of 1 per cent. The first two should be suspended in the hardening and the preserving fluids by means of a glass float, the hook of which has been passed through the margin of the base. Before

beginning operations, see that the specimens of *Cladactis* and *Cereactis* are perfectly sound and especially that they are not torn or cut, otherwise when they are placed in alcohol the liquid contents of the body will exude through the rents. Large specimens of *Cerianthus* are fixed with acetic acid and immediately afterwards are suspended in weak alcohol by means of a thread fastened around the column near the base. A few gentle shakes may be needed to adjust the tentacles. It is not necessary to suspend the small specimens.

Actinia equina and *A. cari* are treated with boiling mixture of sublimate and acetic acid, followed by chromic acid of one-half of 1 per cent for hardening. Frequently success has been attained with the first of these species by lifting it gently with a spatula from the beaker in which it is expanded and immersing it in a saturated solution of sublimate.

Edwardsia is slowly narcotized by dropping from time to time a few drops of 70 per cent alcohol into the sea water in which it is. It is then killed with hot saturated sublimate. Success depends upon the complete loss of sensitiveness, which may be tested by touching the tentacles with a needle point.

Certain species of *Polythoa* are very difficult to prepare. Even with reagents of rapid action they will have the body well distended and often only a portion of the tentacles outside the disk. One species which lives in sponges and among calcareous algæ (probably a variety of *P. axinellæ*) is prepared very successfully with boiling saturated sublimate.

Larvæ of the Actinians are killed with saturated sublimate or with chrom-acetic No. 2.

MADREPORARIA.

Astroides calycularis is allowed to remain overnight in a beaker filled with clear sea water. The following morning usually shows the polyps in full distension. Then, after turning off a portion of the water (enough to leave the animals barely covered), kill them with a solution of boiling sublimate and acetic acid in volume equal to that of the sea water, and immediately afterwards transfer the colony to 35 per cent alcohol, making an injection of the alcohol into each polyp to keep it well distended. At each change of alcohol up to 70 per cent make a similar injection, and be sure to test the final solution with tincture of iodine to see that the sublimate has been eliminated.

Caryophyllia, *Dendrophyllia*, and *Cladocora* are fixed with boiling saturated sublimate, but it is very difficult to prepare them with the polyps in perfect expansion on account of their great contractility and also by reason of the extreme delicacy of their walls.

HYDROMEDUSÆ.

The Hydromedusæ in general are very delicate forms, which are easily injured and which quickly decompose, hence it is necessary to

proceed with their preparation as soon as possible after they have been taken from the sea. Certain Campanularidæ in particular, such as *Aglaophenia*, *Plumularia*, *Sertularia*, and the like, which live in deep water, almost always arrive at the laboratory in a damaged condition or dead. They are more easily injured than other forms by the dredge, the bottom net, or other fishing apparatus. The best plan to follow with such specimens is to put them directly into alcohol, to preserve the perisarc at least. To treat the animals perfectly, they must be attended to on shipboard as soon as caught.

Other forms which live at less depth, and which can be fished by using every precaution against injuring them, must be handled with great rapidity, otherwise, after a short time, the polyps become retracted, and it is very difficult to kill them in an expanded state. In general, these forms are more contractile than the Tubularidæ.

All the Hydroidea—that is to say, the permanently polypoid forms, with very rare exceptions—are killed with hot saturated sublimate. If the polyps are not in complete expansion when received, the colonies should be allowed to expand in beakers of fresh sea water. As soon as the fixing fluid has been poured over the animals the whole should be turned into a crystallizing dish, containing fresh water, to cool; then the animals should be removed to another dish of fresh water for washing, and after five minutes to weak alcohol (50 per cent). If it is desired to avoid the heating of the liquid, cold sublimate can be used, but only for the Tubularidæ.

Large colonies of *Tubularia* and *Pennaria* are killed with the mixture of sublimate and chromic acid in volume equal to that of the water in which they are, and after a few minutes they are washed and removed to alcohol. *Antennularia* may be killed in cold sublimate, washed in fresh water, and placed in 50 per cent alcohol, and so on.

MEDUSA FORMS OF THE TUBULARIDÆ.

The small forms, *Eleutheria* (*Clavatella*), *Cladonema*, *Podocoryne*, and the like, are killed with the mixture of sublimate and acetic acid, used in large proportion. *Eleutheria* may be killed with Kleinenberg's solution.

Lizzia kœllikeri and *Oceania pileata*, as soon as the tentacles have become well distended, are killed with concentrated acetic acid and immediately poured into a tube containing the mixture of alcohol and chromic acid. By gently agitating the tube the animal regains its normal form. After remaining in the mixture about fifteen minutes it is placed in 35 per cent alcohol, and then gradually transferred to that of 70 per cent. Another and perhaps better way of handling these forms is to allow them to expand in a specimen tube less than half full of water, and when they are well distended to fill the tube with acetic acid. Transfer them at once by pouring into the tube containing the mixture of alcohol and chromic acid. A few minutes later pour out a small portion of the liquid in the tube and add chromic acid, because

considerable water will have gone over with the animals. After fifteen minutes wash in fresh water and transfer to 35 per cent alcohol. Still another method is to use the chrom-osmic acid mixture as a hardening medium, but the animals do not remain as transparent and the tentacles are somewhat contracted.

During the hardening process, especially when many of the medusæ are treated at the same time, the tube should be held in a horizontal position in such a way that the bells are down on the side of the tube and the animals not in contact. For the final preservation of certain forms (like *Lizzia*) place them in alcohol in a small tube, separating the individuals by wads of cotton, and put this into the exhibition jar.

Oceania conica and *Tiara pileata* are narcotized in alcoholized sea water (3 per cent) and then treated like the preceding.

MEDUSA FORMS OF THE CAMPANULARIDÆ.

Eucope, *Gastroblasta*, and *Obelia* are fixed in the mixture of sulphate of copper and sublimate and after a few minutes are washed in fresh water until every trace of precipitate has vanished, and then placed in weak alcohol, and so on.

Mitrocoma and *Æquorea* are killed with acetic acid and immediately transferred to the chrom-osmic mixture, where they may lie from fifteen to thirty minutes, according to their size. Small specimens of *Æquorea* can be placed at first in the chrom-osmic mixture.

Tima flavilabris is best preserved by killing in formalin of 4 per cent, where it may remain from five to twenty days, if desired, before the transfer to alcohol begins. This transfer must be made very gradually. The older method of treatment is to kill the animal with chromic acid of 5 per cent in volume equaling that of the water in which it is, and after five minutes to transfer it to the chrom-osmic mixture. After half an hour in the latter mixture wash in fresh water and transfer to alcohol. This method stains the animal brown, while that with formalin leaves it colorless.

Olindias mülleri.—The old method is to kill with acetic acid and immediately transfer to chromic acid of 1 per cent, where the marginal tentacles are to be stretched out by means of a pair of forceps, and where the animals may remain about a quarter of an hour before they are removed to the weak alcohol. The new and more satisfactory method is to place them bottom side up in a shallow tray partly filled with sea water and suddenly pour over them a volume of 6 per cent formalin equal to that of the water in the tray. In the 3 per cent solution of formalin thus made they are to remain at least a week before they are removed to 35 per cent alcohol, and gradually thereafter to 70 per cent alcohol.

Trachymedusæ.—*Rhopalonema*, *Cunina*, *Ægineta*, *Æginopsis*, *Liriope*, and *Carmarina* are fixed in the chrom-osmic liquid for five to twenty minutes, according to their size, washed in fresh water, and gradually

transferred to alcohol. *Cunina* is better when killed with concentrated acetic acid before being hardened with the chrom-osmic mixture. A simpler method for *Carmarina* is to use formalin of 4 per cent and chromic acid in equal parts for killing and hardening. Allow the specimens to remain in this mixture from one to two hours; then wash in fresh water and transfer to alcohol.

To prevent the flattening of the bell of *Carmarina*, *Tima*, and other large forms place a watch glass in the bottom of the jar and rest the bell of the hydrome lusa in its concave side.

ACALEPHÆ.

Charybdæa should be killed with the chrom-acetic mixture No. 2 and immediately afterwards treated with chromic acid of one-half of 1 per cent. After a half hour transfer to alcohol, taking care for the proper suspension of the tentacles.

Nausithoe, the ephyra of *Pelagia*, and *Rhizostoma* are killed by pouring into the sea water containing them 3 per cent of a 1 per cent solution of osmic acid. When they have just begun to take on a brown tint they should be washed in fresh water and placed in 35 per cent alcohol. Formalin of 4 per cent may be used with excellent results with these animals, because it does not give the brown tint which is imparted by osmic acid. To avoid the flattening of the umbrella of *Rhizostoma*, the animal is killed in an exhibition jar with a somewhat narrow neck. After the transfer to weak alcohol the mouth of the jar should be covered with a piece of bladder and should stand upside down, with the convex part of the bell resting in the neck. The medusa should remain in this position until the alcohol has been changed to 70 per cent and the whole body has become permeated with the fluid. When formalin is used for killing and hardening, this inverted position should be maintained from the first.

Pelagia noctiluca should remain in the chrom-osmic liquid about an hour, and then be washed and placed in weak alcohol. In the alcohol the animal should be suspended by threads tied carefully to the extremity of each tentacle without tearing it. See that the bell does not touch the bottom of the jar and let the animal remain thus only until completely hardened.

Cotylorhiza tuberculata (*Cassiopeia*).—Formalin of from 2 to 3 per cent may be used to good advantage for killing and hardening this species. Another method is to treat with osmic acid, as was done with *Rhizostoma*, but when the brown tint begins to appear, a 5 per cent solution of bichromate of potassium should be substituted for the osmic acid and should be renewed after a few days. The animal ought to remain in this reagent for about two weeks, but it can not remain much longer than that without suffering injury. Then remove the object to 35 per cent alcohol. Numerous crystals of a salt are formed on the outside of the animal and a heavy precipitate falls to the bottom of the

receptacle, making it necessary to change the alcohol frequently and to add a few drops of concentrated sulphuric acid thereto.

The larval forms of the Acalephs (*Scyphistoma*, *Strobila*) are killed with hot saturated sublimate. *Strobila* is also well fixed with a mixture consisting of 9 parts of concentrated acetic acid and 1 part of osmic acid of 1 per cent. From this it is quickly transferred to fresh water for washing and put into alcohol.

SIPHONOPHORA.

As with the Hydromedusæ, the preparation of the Siphonophora should be accomplished as soon as possible after capture, and only those specimens should be treated which are in good living condition. Particularly with the Physophoridæ is it true that the whole colony will go to pieces if it remains for a few hours in the same receptacle in which the water has had a sudden change of temperature, though frequently the breaking up does not take place until the colony comes in contact with the fixing fluid. Much care must also be exercised not to shake roughly the vessel which contains the animals before they have been killed. It has often been observed that a trace of an acid or other reagent in the water is enough to destroy the colony. The receiving vessel must be perfectly clean.

Athorybia rosacea, the single representative of the family of Athorybiadæ, which is found in the Bay of Naples, is very rare, and but one specimen has been prepared at the station. That was killed with the mixture of sulphate of copper and sublimate. The colony contracted somewhat, but remained entire. It was washed with fresh water and then placed in alcohol.

The very delicate species (*Physophoridæ*, *Agalmidæ*) must be transferred from the jar in which they were captured to the crystallizing dish in which they are to be killed by immersing both vessels in a tank of water and cautiously pouring the animals over. Leave water enough in the crystallizing dish to give the colony free movement, and wait for the polyps and the nettle-filaments to become well distended and naturally arranged before going on with the treatment.

Physophora, *Agalma*, *Halistemma*, and *Forskalia* are killed with the mixture of sulphate of copper and sublimate in volume equal to or double that of the water in the crystallizing dish containing them. The mixture must be poured rapidly into the dish and not directly onto the animal. After a few minutes, as soon as dead, the colony should be transferred by means of a large horn spatula to the hardening solution, which is not the same for all the species.

(*a*) *Physophora*, *Agalma*, and *Halistemma* are put directly into 35 per cent alcohol, and after a few hours transferred to that of 70 per cent. As soon as *Physophora* has been put into the 35 per cent alcohol, its nettle-filaments should be stretched out as far as possible with a pair of light forceps before they become rigid. To change the liquid in the swimming bell it is necessary to make an injection with a pipette. Bubbles

of air always form in the bells, which, through their tendency to float, tend to change the natural shape of the bells, or, raising the whole colony, flatten it at the surface of the liquid. To get rid of these bubbles it is necessary to compress the bells gently.

(*b*) The genus *Forskalia* is transferred from the mixture of copper sulphate and sublimate to Flemming's solution.[1] The animals remain in this form two to six hours, according to their size, and are then washed in fresh water and transferred to weak alcohol, and so on gradually. The hardening of large colonies succeeds better in the mixture of bichromate of potassium and osmic acid, where they can lie even twenty-four hours without hardening too much. To free the animal from the crystals which form in the tissues and render them opaque, a few drops of concentrated sulphuric acid should be added to the first alcohol into which the colony is put. After that, pure alcohol may be used.

For the permanent preservation of the Physophoridæ, after they have remained for a few days in 70 per cent alcohol in crystallizing dishes for hardening, they are to be put into tubes, arranging them so that the anterior end of the colony is toward the mouth of the tube, by immersing the tube in the liquid and gently working the colony into it. Small specimens of *Agalma* and *Halistemma* can be taken by the posterior end with small forceps and gently forced into a tube filled with 70 per cent alcohol so that the bells point toward the opening. The tube should be small enough to keep the colony in proper position within it. It should be plugged with cotton and placed within a larger tube filled with 70 per cent alcohol and closed with a cork. This double-tube system prevents movement of the liquid about the colony itself, even when the outer tube is not entirely filled with alcohol. It is likewise very useful for shipment of specimens, and especially so for purposes of demonstrations. It is recommended for all very delicate animals and those with appendages which can be injured easily.

Apolemia uvaria is killed as are the preceding species and hardened with 1 per cent chromic acid, which is substituted (in the same dish) for the sulphate of copper and sublimate mixture which has been drawn off through a siphon. After twenty minutes in the acid, wash in fresh water and transfer to alcohol, the change of fluids being effected by means of siphons.

Rhizophysa should be allowed to expand in a beaker with the least practicable amount of water and should then be killed with hot saturated sublimate. Wash at once and put into weak alcohol, rearranging as far as possible the nettle-filaments and tentacles which have become tangled during the handling.

Physalia caravella should be permitted to expand its appendages and polyps in a tall cylinder filled with clear sea water, taking care not to touch the pneumatophores on account of their severe stinging action.

[1] Chromic acid of 1 per cent, 25 c. c.; osmic acid of 1 per cent, 10 c. c.; glacial acetic acid, 5 c. c., and distilled water, 60 c. c.

The preparation succeeds much better in a very high cylinder, on account of the great extensibility of the nettle-filaments. When the colony is well distended pour over it a volume of the sublimate and acetic acid mixture equal to one-fourth that of the sea water containing it. As soon as dead, the colony should be transferred in the same manner as at first to a similar cylinder containing chromic acid of one-half of 1 per cent and after twenty minutes to 50 per cent alcohol, and finally to that of 70 per cent.

Hippopodius, *Galeolaria*, and *Abyla*.—Kill with the mixture of sulphate of copper and sublimate and put directly into weak alcohol. The bell of *Abyla* is also well prepared with the chrom-osmic liquid.

Praya is fixed like *Hippopodius*, but is hardened in the mixture of potassium bichromate and osmic acid, where it may remain one or two days.

Diphyes.—Use hot sublimate for killing, with the chain of the individuals distended.

Velella is killed with the chrom-picric mixture, or with that of sublimate and chromic acid, and after a few minutes is removed to weak alcohol.

Porpita is slowly killed by dropping with a pipette a few drops of Kleinenberg's solution into the beaker where it has become distended. When the beautiful blue color of the colony has begun to turn red as an effect of the acid, it should be removed to the Kleinenberg solution, where it may remain fifteen minutes before it is put into weak alcohol.

CTENOPHORA.

Beroe ovata, *Hormiphora*, *Callianira*, *Lampetia*, *Euchlora*, and young specimens of *Cestus*, *Eucharis*, and *Bolina* are killed in the chrom-osmic mixture, in which they remain from fifteen to sixty minutes, according to size, and are then transferred gradually to 70 per cent alcohol.

While *Beroe ovata* is hardening in the alcohol, insert a short glass tube of the proper size into its gastric cavity to keep it distended in natural shape. Fix the tube so that it acts like a float to keep the animal suspended in the liquid. This operation must be effected with great care to avoid injuring the longitudinal series of vibratile plates. After one or two days, when the animal is in the 70 per cent alcohol, the tube may be removed and the hardened animal will preserve its form.

Beroe forskalii, to be preserved in a state of expansion, must be immersed in the sulphate of copper and sublimate mixture, and as soon as dead must be placed in the chrom-osmic mixture to harden for an hour. Since this species is naturally flat, it is not necessary to introduce a tube into it.

Callianira may be treated like the last species, but another good method is to kill it in a solution composed of—

Concentrated pyroligneous acid	1 part.
Saturated sublimate	2 parts.
Chromic acid of one-half per cent	1 part.

Cestus veneris.—Have the animal in a little water in the exhibition jar and rapidly pour over it enough of chrom-acetic mixture No. 1 to fill the jar three-quarters full, arranging the animal in a coil with the broad edge on the bottom by means of a slender glass rod. After ten minutes substitute chromic-acid solution for the chrom-acetic, and after fifteen minutes therein wash thoroughly in fresh water by decantation and place the animal in 35 per cent alcohol. The gradual transfer to 70 per cent alcohol must take several days, and the jar must not stand in direct sunlight or even strong diffused light. The specimens to be treated should be in perfect condition; otherwise they will go to pieces in the fixing fluid. *Cestus veneris* can be well prepared in the chrom-osmic mixture also, but many specimens are injured and colored too much, whereas by the method just described they remain white and nearly transparent.

Vexillum may be treated like *Cestus veneris.*

ECHINODERMA.

Crinoidea.—Antedon rosacea (*Comatula*) is put directly into 70 per cent alcohol, but *A. phalangium*, on account of its tendency to break in pieces of itself, must be killed in that of 90 per cent. Shake the vessel violently to hasten death and prevent the animals from breaking off their arms. When in doubt about the species use 90 per cent alcohol.

The larval forms of the Pentacrinoids are narcotized with chloral hydrate of 0.1 of 1 per cent—a process requiring two to four hours. If they are then hardened in alcohol, they will remain with the arms perfectly distended. The more advanced stages are best killed with saturated sublimate, where, however, they should remain only a few moments to avoid injury to the membranes.

Asteroidea.—To prepare the Stellarids with the ambulacral feet in a state of distention, they are allowed to die in alcohol of 20 to 30 per cent, being placed in the vessel ambulacra uppermost.

Luidia.—Lay the animal on its back in a shallow tray in barely enough water to cover it. Then, when the ambulacral tentacles, which are very long, are well distended, pour over it the chrom-acetic mixture No. 2 and immediately transfer to 50 per cent alcohol and after two hours remove to 70 per cent alcohol. Small individuals may be killed with 55 per cent acetic acid, but greater care must be used to transfer them to alcohol as soon as dead.

Brisinga easily breaks off its arms, to avoid which it should be quickly immersed in absolute alcohol.

Bipinnaria makes excellent preparations when killed with the chrom-acetic mixture No. 1, and even better with the chrom-osmic mixture, in which it should remain only a few minutes. Other larval forms are treated with saturated sublimate solution.

Some Ophiuroids are allowed to die in fresh water because they thus remain distended and entire. *Ophiothryx echinata* is an example. Certain small forms (*Amphiura*, *Ophiactis*) can be fixed directly in weak

alcohol, shaking the vessel violently to hasten death. *Ophiomyxa pentagona*, which has a soft body, is hardened in chromic acid of one-half per cent. *Ophiopsila annulosa* breaks in pieces of itself in fresh water, and is therefore killed in absolute alcohol.

Echinoidea.—To prepare these with the ambulacral feet well distended they are placed in just sea water enough to cover them and an equal volume of chrom-acetic mixture No. 2 is poured into the jar. They must be transferred at once to alcohol, so as not to give time to the acid to corrode the calcium carbonate of the animal. If desirable to preserve the soft parts of the animal for anatomical purposes, or even for form only, two small holes must be made in the shell opposite each other to discharge all the fluid contained therein. After immersion in alcohol one must see that the liquid fills the internal cavity, and, on changing the animal to stronger alcohol, that the liquid contained within the shell is changed also.

If dry specimens of echini are wished, discharge the water within the shells and let the animals lie in ordinary 70 per cent alcohol for one or two days before placing them in the wind and sun to dry. Starfish also make better dried specimens if they are killed in 70 per cent alcohol and allowed to remain there two or three days before they are dried.

Holothurioidea.—Holothurians require more care than other echinoderms, because they have soft and very contractile bodies and all are furnished with tentacles which contract or retire within the body on contact with a reagent. Some species, furthermore, soon after they are immersed in the fixing fluid expel their viscera and become valueless as specimens—a thing which may happen in the sea water also, if that is changed too suddenly. All these inconveniences are avoided by treating the animals by the methods here described.

First of all, as with other animals which must become expanded, they are placed in clear sea water. Those species which are killed in acids should be allowed to remain in them only just long enough to cause death, so that the calcareous cutaneous spicules be not injured. Large specimens of *Holothuria* and *Stichopus* as soon as the tentacles are fully distended should be seized with two fingers, or with a pair of forceps, a little below the tentacles, lifted from the sea water, and be immersed as to the anterior portion only in a rather deep vessel containing concentrated acetic acid. At the same time another person should inject some 90 per cent alcohol into the animal through the anal aperture with a syringe, taking care not to exert too great pressure, lest the body be distended too much. Before the Holothurian is quite dead it is to be immersed in 70 per cent alcohol, closing the anal orifice with a small cork to prevent the escape of the liquid and the consequent flattening of the body. The injection should be repeated at each successive renewal of the alcohol.

With certain species, as, for example, *Holothuria poli*, the operations must be performed with much caution, because the skin is easily injured.

H. impatiens, which has a long, soft body, is to be seized by the neck, so that the tentacles can not contract, and by the posterior extremity, so that the body may not shorten, and in this manner the whole animal is immersed in concentrated acetic acid. When dead it is transferred at once to alcohol, without making an injection.

Thyone, *Thyonidium*, and *Phyllophorus* are strangled without using much force, wholly immersed in acetic acid, and then removed to alcohol as soon as dead. If the individuals are very small, the pressure on the neck should be made with forceps instead of the fingers.

Cucumaria plancii is treated like the large Holothurians, except that the injection of alcohol is made through the mouth, taking care to keep the tentacles distended, and it is not necessary to close the opening with a cork. The other species of *Cucumaria* are killed in the same manner. It is not necessary to inject the small specimens.

The large *Synapta*, in the preparation of which much difficulty has been experienced on account of its tendency to break to pieces, is killed by immersion in a tube containing sea water and ether in equal parts. In this mixture the animals usually die completely distended. When dead and well distended, they are washed in fresh water and put into weak alcohol. The transfer to 70 per cent alcohol should be very gradual, to avoid contraction. Chloroform may be used instead of ether. The hardening may also be done by putting 2 or 3 c. c. of 1 per cent chromic acid into the water in which they have been washed. After a few seconds remove to weak alcohol.

The rare *Molpadia musculus* and the little *Chirodota venusta* have been also prepared by this method. *Auricularia* is best killed in the mixture of sulphate of copper and sublimate or in sublimate alone.

ENTEROPNEUSTA.

Balanoglossus is killed with the Kleinenberg solution or in chromic acid of one-half of 1 per cent, but the former is much better. When narcotized in alcoholized sea water the animals remain well distended and straight. *Tornaria* is killed with the sulphate of copper and sublimate mixture. It is well preserved also with saturated sublimate or with the chrom-osmic mixture.

VERMES.

The Cestodes are fixed with cold saturated sublimate, the Trematodes with the same solution hot. If it is desired to have flat preparations to mount for the microscope, the animals should be placed between two plates of glass, which are brought together by gradual pressure, and then placed in a crystallizing dish under moderate weights. When the animals are flattened enough and there is as little water as is practicable in the dish, pour over them boiling saturated sublimate and leave them therein until they show no signs of contraction. Then, the plates of glass being removed, the worms are allowed to harden thoroughly in cold saturated sublimate, since the boiling sublimate fixes only the

margins of the objects, not being able to penetrate well between the two plates. In this manner well-distended, flat preparations have been made of *Tristomum*, *Acanthocotyle*, *Distomum*, *Calicotyle*, and of many other Distomata and Polystomata.

Rhabdocœlia and Dendrocœlia.—When they are not quite thoroughly distended in a little water they are killed with boiling saturated sublimate and at once poured into a larger receptacle containing fresh water, where liquid and animals are allowed to cool. From this mixture they are transferred to fresh water, and after a few minutes to alcohol. For certain Polyclades (*Eurylepta*, *Pseudoceros*) it is necessary that the sublimate be warmed a little again, otherwise the bodies break up.

Müller's larvæ may be killed with saturated sublimate, either cold or hot.

Nemertinæ.—Great difficulty has been encountered in dealing with the nemertine worms, because before they are competely distended they contract again, twisting the body badly and breaking to pieces. For some time the effort was made to narcotize the different species by dropping alcohol little by little into the sea water containing the animals, so that as the two mixed the animal would gradually lose sensitiveness and would die. Although this operation was performed with the greatest care, and the worms showed no signs whatever of life, when transferred to the fixing liquid they contracted and became distorted. If the method just described be used with large specimens of *Cerebratulus marginatus*, one can not tell whether the animals are entirely dead or not. Good success, however, has been attained by rapidly plunging them head first into a mixture consisting of Müller's solution[1] 7 parts and concentrated hydrochloric acid 1 part. After a few minutes wash the animals and harden them in alcohol in a wax-bottomed tray.

After repeated experiments the Nemertines were at last successfully narcotized in a solution of chloral hydrate in sea water of 0.1 of 1 per cent strength, where they were allowed to remain from six to twelve hours and then hardened in alcohol in the long zinc box with wax in the bottom. When these animals have been narcotized for not too long a time in the chloral hydrate they will fully regain vitality and power of movement after a little if placed again in sea water.

By this method good preparations can be made of the genera *Carinella*, *Cerebratulus*, *Drepanophorus*, *Nemertes*, *Polia*, etc., in a state of perfect distention and with the proboscis protruded. The more resistant forms (*Langia*, *Amphiporus*, and also *Drepanophorus*), after they have been narcotized in a 0.1 of 1 per cent solution, may well be placed for some hours in a 0.2 of 1 per cent solution before they are killed.

The larval form, *Pilidium*, is killed either with the sulphate of copper and sublimate mixture or with saturated sublimate alone.

[1] Potassium bichromate, 2 grams; sodium sulphate, 1 gram; distilled water, 100 grams.

The Nematodes, free and parasitic, are killed with saturated sublimate or with Kleinenberg's liquid.

Chætognatha.—These are very well treated in the mixture of sulphate of copper and sublimate and in the chrom-osmic mixture.

Gephyrea—Sipunculus is killed with chromic acid of one-half of 1 per cent or even weaker, in which the tentacles usually, but not always, expand before death. The animal, after being narcotized with chloral hydrate of 0.1 of 1 per cent in sea water, dies with tentacles distended. Both methods are good, but sometimes a portion of the animal remains contracted, and sometimes during the process the skin in front breaks and allows all the perivisceral liquid to escape, with resulting distortion of the body.

Phascolosoma may be placed in alcoholized sea water and allowed to remain there until dead (three to six hours). *Phoronis* is allowed to remain half an hour in alcoholized sea water and then is killed with boiling saturated sublimate. With the large specimens of *Bonellia* it is best to wait until the proboscis has become well distended and then seize the body of the animal with one hand and the extremity of the proboscis with a pair of forceps so that it can be kept distended. Then quickly immerse the whole in Kleinenberg's liquid in the wax-bottomed tray, and, always keeping the animal stretched out to prevent contraction, wait until it dies. After lying for an hour in this solution the transfer to alcohol may begin. Small Bonellias are narcotized in alcoholized sea water and fixed in weak alcohol. The very small specimens of these Gephyreans are very well killed with hot sublimate. The pelagic larvæ of *Echiurus* are well fixed by allowing them to lie for some minutes in the mixture of sulphate of copper and sublimate.

Hirudinei, Pontobdella, and *Branchellion* are killed in chromic acid of one-half of 1 per cent. If in doubt about worms of this family, hot saturated sublimate may be recommended for use. In any case long specimens must be straightened and hardened in the wax-bottomed tray.

Chætopoda.—Many of these, if placed in a fixing fluid which is too energetic in action, contract greatly and twist out of shape, and many of them break to pieces, so that an idea of the natural form is lost. This trouble has been obviated by very gradually pouring over the surface of the sea water in the crystallizing dish a stratum of a mixture of glycerin, 1 part, 70 per cent alcohol, 2 parts, and sea water, 2 parts. This stratum will slowly diffuse throughout the water, and after some hours the animals will be narcotized and will remain fully distended, if transferred to alcohol.

Experience at the station has shown that alcohol alone suffices for the treatment of these worms. Instead of the mixture just described, one may add to the sea water 5 per cent of absolute alcohol and let the animals remain therein until they have lost motion, an operation which varies from two to twelve hours for the different species. It is a good plan not to allow these worms to become entirely dead in the

sea water. The hardening is done in the long wax-bottomed trays with 70 per cent alcohol, straightening out the animals and holding them in place when necessary by means of wooden pins. After a few hours in the tray, they should be put into tubes and allowed to rest in a horizontal position for a day or so. Since 70 per cent alcohol does not penetrate the tissues of these animals well enough to prevent maceration, 90 per cent alcohol must be used for permanent preservation. Large specimens should be suspended in the tube by means of a thread attached to a float.

The method just described has given good results with annelids belonging to the following families: Polygordiidæ, Opheliadæ, Capitellidæ, Telethusidæ, Maldanidæ, Ariciidæ, Cirratulidæ, Spionidæ, Terebellidæ, with the exception of the genera *Polymnia* and *Lanice*, which are killed with the mixture of sublimate and chromic acid; among the Aphroditidæ certain Polynoinæ, and all the Sigalioninæ; the Amphinomidæ, which can also be well treated with saturated sublimate; among the Eunicidæ, the Staurocephalinæ, Lysaretinae, and Lumbriconereinæ; all the Nereidæ, Glyceridæ, Syllidæ, Hesionidæ, and Phyllodocidæ.

In the family of the Chlorhæmidæ the genera *Stylarioides* and *Trophonia* are narcotized with alcoholized sea water, hardened in chromic acid of 1 per cent, and transferred to alcohol. *Siphonostomum diplochaitos* of the same family is killed in a solution of chloral hydrate of 5 per cent, and after hardening for fifteen minutes in 1 per cent chromic acid is transferred to alcohol. Another good method is to use the sulphate of copper and sublimate mixture for killing, allowing the animals to remain five minutes in the solution. Animals of this species, when treated with the ordinary reagents, break to pieces with the greatest ease.

Hermionidæ are immersed directly in 70 per cent alcohol (old solution will do), taking care that the animals do not die in a curved position.

Chætopteridæ, Sternaspidæ, the large *Spirographis* and the large Serpulinas of the genus *Protula* are killed in 1 per cent chromic acid, where they are allowed to rest at least half an hour. Then after thorough washing in fresh water, they are put into alcohol of 70 per cent, and afterwards into that of 90 per cent. *Spirographis* can not be well treated in its tube or be returned to it after treatment. *Myxicola infundibulum* is killed in saturated sublimate, and, after ten or fifteen minutes, thoroughly washed and put into 50 per cent alcohol for a few hours before it is put permanently into that of 70 per cent.

The following annelids are killed with cold saturated sublimate, in which they should not be allowed to remain more than fifteen minutes; all the Amphictenidæ (which may be placed in alcoholized sea water until well out of their tubes), the Hermellidæ, the Serpulidæ (some of which should remain for some hours in a 0.1 of 1 per cent solution of chloral, so that they may come wholly or partly out of their tubes), of the

Aphroditidæ certain Polynoinæ, *Polyodontes maxillosus*, of the Eunicidæ all the group of the Eunicinæ. Some of these, like *Dioptara*, are best fixed by narcotizing them in alcoholized sea water.

Alciopidæ are very well prepared by letting them die in the sulphate of copper and sublimate mixture. They should remain in the solution not to exceed five minutes, and then be washed thoroughly in fresh water before they are placed in alcohol.

Tomopteridæ are preserved in the way just described, or with cold saturated sublimate. Remove the last traces of sublimate with iodine.

CRUSTACEA.

The marine Cladocera (*Podon*, *Evadne*) are killed with saturated sublimate, or with a few drops of osmic acid of 1 per cent in the sea water containing them, removing them when they begin to turn brown. Wash and put into 70 per cent alcohol.

Ostracoda are put at once into 70 per cent alcohol.

Copepoda.—The free forms are killed in a saturated solution of sublimate in sea water, where they are allowed to stay from five to ten minutes. The parasitic forms may be killed in the same way or be put at once into weak alcohol.

Cirripedia.—To prepare *Lepas*, *Conchoderma*, etc., with the cirrhi distended, let them die in alcohol of 35 per cent. If the cirrhi of certain species contract they can easily be drawn out again by means of forceps.

Balanus and similar forms are immersed directly in alcohol of 70 per cent, taking care to change the liquid soon.

Rhizocephala (*Sacculina*, *Peltogaster*, etc.) are placed for fifteen minutes in a mixture of 90 per cent alcohol and sublimate in equal parts, and then transferred to 70 per cent alcohol.

Amphipoda.—All the Læmodipodes, Crevettines and Iperines were formerly prepared by putting them at once into alcohol of 70 per cent, but now they are put into Perenyi's solution for fifteen minutes before they are immersed in the alcohol. The transparent forms of the last division (*Phronima*, etc.) are killed in sublimate.

Isopoda.—These are put at once into 70 per cent alcohol, with the exception of the *Bopyrides* and *Entoniscides*, which are first placed in a mixture of 90 per cent alcohol and saturated sublimate in equal parts (like the Rhizocephala) or in saturated sublimate alone.

Cumacea and *Stomatopoda* go directly into alcohol, though the transparent larval forms of the latter are first put into saturated sublimate for a few minutes.

Schizopoda go at once into alcohol or they may be treated first with saturated sublimate.

To avoid the breaking of the appendages of the *Decapoda*, these forms are allowed to die in fresh water before they are put into alcohol. They remain in fresh water only as long as is necessary, otherwise the membranous appendages are injured.

With the Paguridæ the alcohol must be changed often, and they are to be preserved permanently in 90 per cent alcohol, because the shell is permeable to only a small degree.

The larvæ of the Decapods (*Zoëa*, *Phyllosoma*, etc.) are killed in sublimate or with a few drops of osmic acid of 1 per cent.

PANTOPODA.

The Pantopoda are killed in chromic acid of one-half per cent so that they will remain with the legs distended. As they are almost always covered with foreign bodies, it is necessary to let them live for several days in beakers of fresh sea water, so that they may clear themselves of these extraneous growths.

MOLLUSCA.

Lamellibranchs may be prepared with the valves open by narcotizing them in alcoholized sea water, where they may remain from six to twelve hours or even more, according to the species. The siphonate forms should not be transferred to alcohol before they are thoroughly stupefied, otherwise the siphons will contract. After the animals have been narcotized, it is an excellent plan to place them in chromic acid of 1 per cent for about a half hour. They are very likely to open their shells wider after they have been placed in the chromic acid. When chromic acid is not used, it is well to place a bit of wood between the valves to keep them apart when first put into alcohol. Cocaine may be used instead of alcoholized sea water for narcotizing the animals. The animals are preserved in a more distended condition, but its use is not as necessary for lamellibranchs as for gastropods, and the method will be described when the latter are discussed.

Lima, which has a large number of tentacular filaments around the edge of the mantle, which break off if alcoholized sea water be used, is killed with chromic acid of one-fourth of 1 per cent. Large specimens, however, yield better results if treated with the copper sulphate solution first.

Scaphopoda.—*Dentalium* is narcotized with chloral hydrate of 0.2 of 1 per cent, in which it remains from ten to twelve hours or more, and is then put into 70 per cent alcohol.

Gastropoda.—The use of cocaine for narcotizing all species of gastropods is strongly recommended. The solution consists of 2 grams of cocaine powder dissolved in 100 c. c. of 50 per cent alcohol. Place the animals in the least practicable amount of water. Drop in a few drops of the cocaine solution, and after two hours add a few more, and continue the operation until the animals are thoroughly insensible. The action is much slower in winter than in summer. To avoid the contraction into the shell, which is apt to take place with prosobranchs having a spiral shell, even when the narcotizing has seemed to be complete, draw the operculum as far out as possible with a pair of pincers and bind it to the shell.

As cocaine is not always available, the old methods for treating gastropods will be detailed. It is to be understood that when cocaine is used for narcotizing the subsequent treatment is that indicated below:

The Placophora and the families of the Patellidæ, the Fissurellidæ, and the Haliotidæ may be prepared in a distended condition by narcotizing them with alcoholized sea water.

Natica josephinia may be fixed in complete distention by dropping 70 per cent alcohol little by little into the sea water until the animals no longer respond to any stimulus, an operation which often lasts two or three days. Then they are killed by rapidly pouring concentrated acetic acid over them, and they are transferred at once to weak alcohol. If one desires to get perfect specimens he must treat several at once, because out of every lot some are sure to remain more or less contracted.

Natica millepunctata and *N. hebrea*, when treated in the manner just described, remain entirely contracted. Good results may be obtained, however, by letting them remain for some days in a mixture of sea water and fresh water in equal parts, and afterwards killing them with acetic acid. This may be used for preparing several species of *Nassa*, *Columbella*, *Conus*, and *Trochus*.

Heteropoda.—The Atlantidæ may be narcotized in alcoholized sea water, where they are allowed to remain for from six to twelve hours, and are then placed directly in alcohol. Cocaine, however, is much better for narcotizing.

The Pterotrachaidæ are killed by immersing them in chrom-acetic mixture No. 1 for from ten to thirty minutes according to their size. Wash thoroughly in fresh water and transfer gradually to the different grades of alcohol. These animals are well prepared also with the chrom-osmic mixture, and the little specimens of *Carinaria* are best treated with the mixture of sulphate of copper and sublimate. Large specimens should be suspended in the permanent receptacle by a thread tied around the end of the proboscis.

Opisthobranchiata.—The Bullas are slowly narcotized in the mixture of sea water and fresh water in equal parts or in alcoholized sea water and allowed to remain therein until thoroughly insensible. They are killed with concentrated acetic acid and transferred at once to alcohol.

Gastropteron meckeli is killed in Kleinenberg's solution, thereby retaining its natural red color very well. It loses its color in the ordinary liquids.

Doridium and Scaphander.—Narcotize in alcoholized sea water, kill in concentrated acetic acid, and quickly transfer to alcohol. If not hard enough, or if softened at all in the acetic acid, they may be placed in chromic-acid solution of 1 per cent for ten or fifteen minutes before they are put into alcohol.

Philine.—When the animal is well distended in a little sea water, suddenly pour over it concentrated acetic or pyroligneous acid and quickly transfer to alcohol.

Pleurophyllidia is narcotized with alcoholized sea water and then killed with concentrated acetic acid.

Aplysia limacina and *A. punctata* are fixed in 1 per cent chromic acid, where they are allowed to remain from fifteen to sixty minutes, according to their size. *A. depilans* is narcotized in chloral hydrate solution of 1 per cent (which may take twelve hours), killed with concentrated acetic acid, transferred at once to chromic acid of 1 per cent, and after half an hour put into 50 per cent alcohol, and so on.

Pleurobranchia meckeli is best treated with cocaine and then put into chromic acid of 1 per cent, where the animals may remain about an hour before they are washed and put into alcohol.

Pleurobranchus meckeli and *P. testudinarius* may be killed in chromic acid of 5 per cent. When scarcely dead the animals are transferred to that of 1 per cent, where they may remain from fifteen to sixty minutes, according to their size. The small specimens can be well prepared with chloral hydrate, also, afterwards fixing them with chromic acid of 1 per cent.

Umbrella is slowly killed in alcoholized sea water, after which it is put into weak alcohol.

The Elysiidæ and the Æolidiidæ are permitted to expand in the least practicable amount of sea water. They are then killed by rapidly pouring over them a volume of concentrated acetic acid double or equal to that of the sea water, and when scarcely dead they are transferred to weak alcohol.

Phyllirrhoe bucephalum is fixed in the chrom-osmic mixture for a few minutes or in the chrom-acetic mixture No. 2.

Doris, *Chromodoris*, *etc.*—The larger specimens of these animals may be narcotized by adding 70 per cent alcohol, a little at a time, to the water containing them, until touching the branchial appendages on the back produces no contraction. They should then be killed with concentrated acetic acid or boiling saturated sublimate. If cocaine is used for narcotizing the animals, they should be killed in concentrated acetic acid, placed in chromic acid of 1 per cent for ten minutes and then transferred to 50 per cent alcohol, and so on. The small specimens need not be narcotized.

Triopa, *Idalia*, and *Polycera* are treated like the Elysiidæ.

The large specimens of *Tritonia* are immersed until dead in fresh water, to which a few drops of acetic acid have been added, when they are hardened in chromic acid of one-half of 1 per cent. By this method they remain well distended and the shape suffers no alteration. Small Tritonias are treated with cocaine and then hardened and placed in alcohol.

Marionia is narcotized in alcoholized sea water and killed in acetic acid.

To prepare *Tethys* with the dorsal appendages in position, the animal is allowed to expand in a large, low crystallizing dish in the least amount of water possible necessary to cover it. It is killed by pouring

over it a quantity of concentrated acetic acid at least as great as the water in the dish. Then the liquid is removed by means of a siphon and chromic acid of 1 per cent substituted therefor. Then carefully try to give the animal a lifelike appearance by flattening the foot on the bottom of the receptacle and arranging the cephalic lobe so that it rests easily rolled up in conical shape. In this manner it should harden, and after half an hour the chromic acid should be siphoned off and weak alcohol introduced. The animal must be suspended in the final receptacle.

Pteropoda.—The Hyaleidæ are placed in a little water and allowed to expand the two wings, when saturated sublimate solution is poured over them. After a few minutes they are washed and placed in weak alcohol, and so on. *Criseis acicula* is well prepared by killing it with alcoholized sea water.

Cymbuliidæ are very well killed in Perenyi's solution, where they may remain fifteen minutes before they are transferred to 50 per cent alcohol. If they are prepared with the chrom-osmic mixture, their form is perfectly preserved, but the transparency is partly lost.

The *Gymnosomata* are placed in chloral hydrate solution of 0.1 of 1 per cent for from six to twelve hours and then are quickly killed with acetic acid or sublimate. Good preparations of *Cliopsis* have been made by letting the animals die in chromic acid of one-fourth of 1 per cent.

Cephalopoda.—The preparations are much better, of course, when the animals are immersed in the preserving fluid while still alive. If they have been dead for some time when received, they can be made to regain their shape in part by allowing them to lie in sea water for about an hour. Then they had better be hardened in 1 per cent chromic acid for fifteen to sixty minutes, according to their size.

Small octopods are narcotized in chloral hydrate of 0.2 of 1 per cent, and then immersed in alcohol, where they sometimes contract, twisting the arms about the body, but after the animals are dead it is easy to stretch out the arms and dispose them in a natural position. The larger animals (of a length of 15 cm. (6 inches) or more) are killed in 1 per cent chromic acid, where they are usually kept a half hour, though very large ones may remain even so much as two hours. After washing them in fresh water, transfer to 70 per cent alcohol, taking care to change the latter several times.

Ocythœ catenulata (*Philonexisa*).—Females of medium size are immersed directly in 70 per cent alcohol and after they are dead their arms are straightened out. *Scœurgus tetracirrhus* (*Octopus*) is killed in the mixture of alcohol and chromic acid and after twenty minutes is transferred to alcohol.

The Decapods may be put at once into alcohol of 70 per cent, taking care, before they are quite dead, to pull out the two tentacular arms, which usually have contracted. The small species should be narcotized in chloral hydrate of 0.2 of 1 per cent or in alcoholized sea water before they are put into the alcohol. To facilitate the penetration of

the alcohol into the visceral region of the largest specimens an incision should be made in the ventral part of the body.

The transparent pelagic forms (*Loligopsis*, *Verania*) are put first into Kleinenberg's solution and after an hour are transferred to weak alcohol, from which they are gradually changed to that of 70 per cent. The forms which are contained in a common gelatinous substance are fixed with chromic acid of one-half of 1 per cent and then transferred to 50 per cent alcohol, where they remain permanently.

BRYOZOA.

Bryozoa are best preserved when they are treated on board ship immediately after they have been caught.

Pedicellina and *Loxosoma* are left for an hour in chloral hydrate of 0.1 of 1 per cent and then killed with saturated sublimate, cold or hot. Wash immediately afterwards and place in alcohol. Certain species of *Bugula* (*purpurotincta*, *turbinata*), after the animals have become well distended in a little sea water, are killed quickly with hot sublimate. By pouring some 70 per cent alcohol slowly over the surface of the water in which they are, *Flustra*, *Cellepora*, *Crisia*, *Bugula*, and *Zoobotrium* have been prepared in a state of complete distention. The other species can be killed with the animals more or less out of their cells by using a weak solution of chloral hydrate or alcoholized sea water, but generally good results depend upon the skill of the preparator.

BRACHIOPODA.

Brachiopods are narcotized by letting them lie in alcoholized sea water for some hours before they are put into alcohol. Put a bit of cork between the valves to keep them open. Small forms may be put at once into 70 per cent alcohol.

TUNICATA.

The Appendiculariæ are killed by letting them rest for five minutes in the chrom-osmic mixture.

Ascidiæ simplices.—To treat *Clavellina* and *Perophora* so that the orifices shall remain open, first allow them to expand in running sea water and then immerse them in chloral hydrate of 0.1 of 1 per cent, letting them remain from six to twelve hours; then kill them with chrom-acetic No. 2 and immediately afterwards transfer to chromic acid of 1 per cent, some of which should be injected into the interior of each individual. After a half hour transfer the animals to 35 per cent alcohol, repeating the injection with that fluid, and finally to alcohol of 70 per cent. *Clavellina rissoana* is usually killed in acetic acid; it is not necessary to inject each individual.

Ascidia (*Phallusia*), after from three to six hours in chloral hydrate of 0.1 of 1 per cent, is hardened for half an hour in chromic acid of 1 per cent, washed, and transferred to alcohol.

Ciona intestinalis is killed slowly by putting into the water in which the animals have expanded a few drops of the chrom-acetic mixture No. 2. When the animal has died, which happens in about a half hour, it is to be taken by the anterior orifice, to avoid the discharge of the water within, and put into chromic acid of 1 per cent, making an injection of the same into the body cavity. The transfer to the alcohol series should be made in the same way.

Certain ascidians (*Ascidia* and *Rhopalea*) are killed in the following manner, so as to keep the orifices open: They should be placed in beakers with from 4 to 5 cm. (about 2 inches) of sea water above them. Then slowly drop in 1 per cent chromic acid in such a manner as to form a stratum on the top of the water. Little by little the chromic acid will diffuse through the water, usually killing the animals in from twelve to twenty-four hours. If the animals are not dead by that time, add a little more chromic acid. Harden in chromic acid of 1 per cent, wash in fresh water, and put into alcohol. The animals should not rest against the sides of the beaker during narcotization. If the animal has not a good base upon which to stand, some clean sand may be placed in the bottom of the glass, in which it can be arranged in the desired position.

Molgula, *Polycarpa*, *Rhopalea*, and *Chevreulius* (*Rhodosoma*) must remain for twelve hours in chloral hydrate of 0.1 of 1 per cent. Then kill them in chrom-acetic No. 2 and transfer at once to 1 per cent chromic acid for a little time to harden.

Cynthia and *Styela* are narcotized in 0.2 of 1 per cent chloral hydrate for twenty-four hours and then treated like the last-mentioned genera. *Cynthia papillosa*, however, sometimes contracts greatly when immersed in chloral hydrate of 0.2 of 1 per cent. When it does, put it back into running sea water to expand again, and then try treating it with 0.1 of 1 per cent chloral hydrate.

Ascidiæ compositæ.—The gelatinous forms—for example, the Botryllidæ, *Polycyclus*, *Circinalium*, and *Fragarium*—are allowed to lie in chloral hydrate of 0.1 of 1 per cent for a few days and then are killed by pouring hot saturated sublimate over them. Immediately afterwards they are transferred to chromic acid of one-half of 1 per cent, where they are left for a half hour before they are washed and transferred to alcohol.

Distaplia, after it has been narcotized with 0.1 of 1 per cent chloral, is killed with chrom-acetic No. 2, washed, and put directly into weak alcohol.

Diazona violacea should remain twelve hours in chloral hydrate of 0.2 of 1 per cent, and then the killing and the hardening should be done as with the Botryllidæ, except that the individual animal should be injected with the liquid. Small colonies may be killed in acetic acid and hardened in one-half per cent chromic acid.

Leptoclinum and other forms of a certain consistency are transferred from the chloral directly to the alcohol.

Pyrosoma is suspended by a thread in the mixture of alcohol and hydrochloric acid in a cylindrical jar, and, after a quarter of an hour, is transferred to 60 per cent alcohol and gradually to that which is stronger. Good preparations have been made by putting the colony directly into 50 per cent alcohol. Care must be exercised to get rid of the minute air bubbles which are apt to form on the surface of the colony, though they usually disappear of themselves.

The Salpidæ include animals of very various consistency, from slimy to cartilaginous. Certain species, furthermore, although they have consistency when young, become soft in the adult stages and difficult to preserve. Sometimes the Salpas when immersed in the fixing fluid contract greatly, closing the orifices and dying in this condition. This may be remedied by introducing a closed glass tube into one of the openings, which, by allowing the entrance of the liquid, causes the animal to resume its natural shape.

The species with a hard body (*Salpa bicaudata* when solitary and young; *S. tilesi*, both chain and solitary forms; *S. zonaria*, both chain and solitary forms), are immersed in a mixture of fresh water (100 c. c.) and concentrated acetic acid (10 c. c.), where they remain for fifteen minutes. Then they are washed in fresh water for ten minutes, and then transferred gradually to alcohol, where it is necessary to float the larger forms by means of pieces of cork attached to them with threads so that the gelatinous sac shall not flatten down upon the intestinal nucleus within. When treated in this manner, the animals remain very transparent, crystals of marine salts forming in the tissues much less than with the other liquids.

The forms of medium consistency (young chains and solitary individuals of *Salpa maxima* and *S. pinnata*, young chains of *S. bicaudata*, both the adult forms of *S. fusiformis* and *S. democratica-mucronata*) are placed in the chrom-acetic mixture No. 1 for ten minutes and then put directly into weak alcohol.

The very soft forms (large chains of *Salpa bicaudata* and *S. punctata*, both forms of *S. maxima*, *S. pinnata*, and *S. virgola*) are immersed in the chrom-osmic mixture for from fifteen to sixty minutes, according to their size. Then they are washed in fresh water and transferred to weak alcohol.

Very large specimens of *Salpa maxima* flatten out of their own weight when put into weak alcohol. This may be obviated by blowing a few bubbles of air into the cavity of the animal or by putting therein a tube of thin glass closed at both ends to act as a float. The tube or the bubbles of air should be removed before the animal is entirely hardened.

Professor Todaro, to preserve Salpas for histological purposes, immerses them at first in Kleinenberg's solution, and after an hour transfers them to alcohol. When preserved in this manner, however, all but the hard species lose their form entirely.